IN THE CLAIMS

Please amend the claims as follows.

1-34. (Canceled)

35. (Withdrawn, Currently Amended) A method to detect or determine the presence or amount of a mutant hydrolase, comprising:

a) contacting a mutant hydrolase with a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the wild-type hydrolase is a dehalogenase, wherein the mutant hydrolase is a mutant dehalogenase, and wherein the substrate is a compound of formula (I): linker R-linker-A-X, wherein the linker is a divalent branched or unbranched carbon chain comprising from ~~about~~ 2 to ~~about~~ 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is $(CH_2)_n$ and n = 2-10 ~~4-10~~, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker; and

b) detecting or determining the presence or amount of the functional group, thereby detecting or determining the presence or amount of the mutant dehalogenase ~~hydrolase~~.

36. (Withdrawn, Currently Amended) The method of claim 35 wherein the substitution is at a residue in the wild-type ~~hydrolase~~ dehalogenase that activates the water molecule.

37. (Withdrawn, Currently Amended) The method of claim 36 wherein the residue in the wild-type ~~hydrolase~~ dehalogenase that activates the water molecule is histidine.

38. (Withdrawn, Currently Amended) The method of claim 35 wherein the substitution is at a residue in the wild-type ~~hydrolase~~ dehalogenase that forms an ester intermediate with the substrate.

39. (Withdrawn, Currently Amended) The method of claim 38 wherein the residue in the wild-type ~~hydrolase~~ dehalogenase that forms an ester intermediate with the substrate is aspartate.

40. (Withdrawn, Currently Amended) A method to isolate a molecule, cell or subcellular organelle of interest in a sample, comprising:
a) contacting a sample with a fusion protein comprising a mutant hydrolase and a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the fusion protein comprises a protein which binds a molecule, cell or subcellular organelle of interest, wherein the wild-type hydrolase is a dehalogenase, wherein the mutant hydrolase is a mutant dehalogenase, and wherein the substrate is a compound of formula (I): R-linker-A-X,

wherein the linker is a divalent branched or unbranched carbon chain comprising from ~~about~~ 2 to ~~about~~ 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is $(CH_2)_n$ and n = 2-10 ~~4-10~~, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker; and

b) isolating the molecule, cell or subcellular organelle of interest.

41. (Withdrawn, Currently Amended) The method of claim 40 wherein the substitution is at a residue in the wild-type ~~hydrolase~~ dehalogenase that activates the water molecule.

42. (Withdrawn, Currently Amended) The method of claim 41 wherein the residue in the wild-type ~~hydrolase~~ dehalogenase that activates the water molecule is histidine.

43. (Withdrawn, Currently Amended) The method of claim 40 wherein the substitution is at a residue in the wild-type ~~hydrolase~~ dehalogenase that forms an ester intermediate with the substrate.

44. (Withdrawn, Currently Amended) The method of claim 43 wherein the residue in the wild-type ~~hydrolase~~ dehalogenase that forms an ester intermediate with the substrate is aspartate.

45. (Canceled)

46. (Withdrawn) The method of claim 40 wherein the molecule of interest is a protein.

47. (Withdrawn, Currently Amended) A method to label a cell, comprising:

a) contacting a cell comprising a mutant hydrolase with a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves a bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the wild-type hydrolase is a dehalogenase, wherein the mutant hydrolase is a mutant dehalogenase, and wherein the substrate is a compound of formula (I): R-linker-A-X, wherein the linker is a divalent branched or unbranched carbon chain comprising from ~~about~~ 2 to ~~about~~ 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is $(CH_2)_n$ and n = 2-10 ~~4-10~~, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker; and
b) detecting or determining the presence or amount of the functional group.

48. (Withdrawn, Currently Amended) The method of claim 47 wherein the substitution is at a residue in the wild-type ~~hydrolase~~ dehalogenase that activates the water molecule.

49. (Withdrawn, Currently Amended) The method of claim 48 wherein the residue in the wild-type ~~hydrolase~~ dehalogenas that activates the water molecule is histidine.

50. (Withdrawn, Currently Amended) The method of claim 47 wherein the substitution is at a residue in the wild-type ~~hydrolase~~ dehalogenase that forms an ester intermediate with the substrate.

51. (Withdrawn, Currently Amended) The method of claim 50 wherein the residue in the wild-type ~~hydrolase~~ dehalogenase that forms an ester intermediate with the substrate is aspartate.

52-54. (Canceled)

55. (Withdrawn, Currently Amended) The method of claim ~~52~~ 35, 40 or 47 wherein the linker comprises $(CH_2CH_2)_y$ and y = 2-8.

56. (Withdrawn, Previously Presented) The method of claim ~~52~~ 35, 40 or 47 wherein the linker separates R and A by at least 12 atoms.

57. (Canceled)

58. (Withdrawn, Currently Amended) The method of any one of claims 35, 40 or 47 wherein the mutant ~~hydrolase~~ dehalogenase is present in a cell or on the surface of a cell.

59-63. (Canceled)

64. (Withdrawn, Currently Amended) The method of any one of claims 35, 40 or 47 wherein the presence of at least one functional group in a cell is correlated to the subcellular location of the mutant ~~hydrolase~~ dehalogenase.

65. (Withdrawn, Currently Amended) The method of any one of claims 35, 40 or 47 wherein the mutant ~~hydrolase~~ dehalogenase further comprises a protein of interest, thereby yielding a fusion protein.

66. (Withdrawn) The method of claim 65 wherein the protein of interest is a selectable marker protein, membrane protein, cytosolic protein, nuclear protein, structural protein, an enzyme, an enzyme substrate, a receptor protein, a transporter protein, a transcription factor, a channel protein, a phospho-protein, a kinase, a signaling protein, a metabolic protein, a mitochondrial protein, a receptor associated protein, a nucleic acid binding protein, an extracellular matrix protein, a secreted protein, a receptor ligand, a serum protein, an immunogenic protein, a fluorescent protein, or a protein with reactive cysteine.

67. (Withdrawn, Currently Amended) The method of claim 47 wherein the mutant ~~hydrolase~~ dehalogenase further comprises a selectable marker protein.

68. (Canceled)

69. (Withdrawn, Currently Amended) The method of claim ~~68~~ 67 wherein the mutant ~~hydrolase~~ dehalogenase forms an ester bond with the substrate.

70. (Withdrawn, Currently Amended) The method of claim ~~68~~ 67 wherein the mutant ~~hydrolase~~ dehalogenase forms a thioester bond with the substrate.

71. (Withdrawn) The method of claim 47 further comprising contacting the cell with a fixative prior to or after contacting the cell with the substrate.

72. (Withdrawn) The method of claim 47 further comprising contacting the cell with a fixative concurrently with contacting the cell with the substrate.

73. (Withdrawn) The method of claim 71 or 72 wherein the cell is fixed with methanol, acetone and/or paraformaldehyde.

74. (Withdrawn) The method of claim 67 further comprising contacting the cell with a fixative prior to or after contacting the cell with the substrate.

75. (Withdrawn) The method of claim 67 further comprising contacting the cell with a fixative concurrently contacting the cell with the substrate.

76. (Withdrawn) The method of claim 74 or 75 wherein the cell is fixed with methanol, acetone and/or paraformaldehyde.

77. (Withdrawn) The method of claim 52 wherein the mutant dehalogenase is encoded by a nucleic acid sequence which is optimized for expression in a selected host cell.

78-106. (Canceled)

107. (Currently Amended) A method for preparing a compound of formula R-Linker-A-X comprising coupling a compound of formula R-Y with a compound of formula Z-Linker-A-X, wherein Y and Z are groups that can react to link R- to –Linker-A-X, wherein the linker is a divalent branched or unbranched carbon chain comprising from ~~about~~ 2 to ~~about~~ 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is $(CH_2)_n$ and n = 2-10 ~~4-10~~, wherein X is a halogen, ~~and~~ wherein R is a biotin functional group that is capable of being coupled through its carboxy terminus to the linker, and wherein R-Y is an activated ester of a compound of formula R and wherein Z is an amine suitable to react with the activated ester to form an amide bond.

108. (Canceled)

109. (Original) A method for preparing a compound of formula R-Linker-A-X wherein the Linker comprises an amide bond comprising coupling a corresponding activated ester with a corresponding amine to provide the compound of formula R–Linker-A-X.

110. (Currently Amended) ~~The compound of claim 1~~ A compound of formula (I): R-linker-A-X, wherein R is one or more functional groups, wherein the linker is a divalent branched or unbranched carbon chain comprising from 2 to 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A is $(CH_2)_n$ and n = 2-10, wherein A-X is a substrate for a dehalogenase, and wherein X is a halogen, wherein R is a biotin functional group coupled through its carboxy terminus to the linker.

111. (Previously Presented) The compound of claim 110 which is a substrate for a *Rhodococcus* dehalogenase.

112. (Previously Presented) The compound of claim 110 wherein X is Cl or Br.

113. (Previously Presented) The compound of claim 110 wherein the linker comprises 3 to 30 atoms.

114. (Previously Presented) The compound of claim 110 wherein the linker has 11 to 30 atoms.

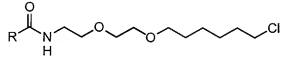
115. (Previously Presented) The compound of claim 110 which is N-{2-[2-(6-Chlorohexyloxy)-ethoxy]-ethyl}-biotin-amide.

116. (Previously Presented) The compound of claim 110 wherein R is separated from A-X by up to 100 angstroms.

117. (Previously Presented) The compound of claim 110 wherein R is separated from A-X by up to 500 angstroms.

118. (Previously Presented) The compound of claim 110 wherein the chain comprises $(CH_2CH_2O)_y$ and y = 2-8.

119. (Previously Presented) A compound prepared by the method of claim 107 wherein the compound is



120. (Currently Amended) A compound of formula (I): R-linker-A-X, wherein R is one or more functional groups, wherein the linker is a divalent branched or unbranched carbon chain comprising from ~~about~~ 2 to ~~about~~ 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A is $(CH_2)_n$ and n = 2-10, wherein A-X is a substrate for a dehalogenase, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker.